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CIRCULATING MICRO-RNA AS A BIOMARKER IN DIABETES MELLITUS

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ABSTRACT
MicroRNAs have been a hotspot in recent years as a biomarker for various diseases such as cancer, bone diseases, myocardial infarction, obesity etc. This article discusses about their role as biomarker in Type 1 and Type 2 diabetes mellitus. Various research has been performed which clearly indicate their role as promising biomarkers. They have also been linked to diabetic retinopathy as well as gestational diabetes. If worked upon they may prove as an excellent biomarker not only for the known diseases but also more of them. The articles discussed here provide convincing data related to the potential of circulating microRNAs.

KEYWORDS: MicroRNAs, Biomarker, Diabetes Mellitus.

INTRODUCTION
MicroRNAs belong to a class of highly conserved, sequence-specific, single stranded endogenous small non-coding RNAs (18-25 nucleotides in length). They have been shown to regulate eukaryotic gene expression by binding to the 3'-end of target mRNAs to induce their instability, degradation and/or translational inhibition.[1] miRNAs can regulate several genes and are involved in several important signalling pathways.

MicroRNA is detectable in various biological fluids such as blood, urine, tears, saliva and cerebrospinal fluid, amniotic fluid or synovial fluid.[2] Unlike other RNA molecules, an important feature of miRNAs is their stability and resistance to external factors such as RNAase.[3] This is due to the form in which they are present in biofluids. MiRNA forms complexes with lipoproteins or proteins. In addition, the protective effect may be due to their entrapment in membrane structures such as exosomes, microparticles or apoptotic bodies.[4] Repeated freeze–thaw cycles have also been shown not to cause significant changes in serum miRNA concentrations.[5]

These mechanisms and non-invasive collection mean that circulating miRNAs have good potential as biomarkers. MiRNAs have broad biological effects because most mRNAs are conserved miRNA targets (miRBase. org), and as a result, miRNAs have the ability to regulate a wide variety of biological processes, including pathological mechanisms.[6,7] A more recent finding is that miRNAs are released into the extracellular space not only as by-products of cell loss/damage, but also as mediators of cell-to-cell communication at a paracrine level and between different tissues.[8]

Outside cells, their stability is ensured by their association with various protective cellular components, such as protein complexes or extracellular vesicles.[9-12] On the other hand, dysregulation of circulating miRNAs in blood may reflect tissue-specific dysfunction; on the other hand, these extracellular miRNAs are capable of orchestrating remote regulatory mechanisms. In other words, they can be used both as new disease biomarkers and as therapeutic targets.[13,14,15]

Diabetes is one of the oldest diseases known to mankind. Over the millennia, type 2 diabetes mellitus (T2DM) has become an epidemic affecting more than four hundred million people worldwide. Diabetes currently affects approximately 415 million people worldwide, which is likely to increase to 642 million by 2040 (IDF). The metabolic disorder is defined as a form of non-insulin-dependent diabetes characterized by hyperglycemia mainly due to the inability of the body to produce and respond to insulin. It is a multifactorial disease, the prevalence of which is due to several factors.

The causative factors have increased due to urbanization caused by sedentary lifestyles, physical inactivity and unhealthy eating habits, especially in developing countries.

This disease is associated with severe, disabling, long-term complications, including heart disease, kidney failure, neurological disease and eye disease, and has become one of the leading causes of morbidity and mortality worldwide.

To design an adequate prevention of T2DM, evaluation of traditional biomarkers, mainly small non-coding RNAs and especially microRNAs (miRNAs) have recently emerged as key regulators of metabolism and metabolic disorders. Growing evidence also implicates microRNAs in the progression of diabetes, which are a class of small non-coding RNAs 20-24 nucleotides long that can regulate the expression of thousands of genes.[15]

Circulating MicroRNA as biomarker

Micro RNAs act as repressors of translation and regulators of important cellular processes. Traditional biomarkers in the form of micro RNA, which are released by most body cells and reach the blood stream in a very stable form, can be used to a distance.[16]

Additionally, several circulating micro RNAs have been reported to be involved in beta cell activity, differentiation and both normal and disease states.[17] Assessment of traditional biomarkers can then be used to identify subjects who already have metabolic changes, such as higher than normal glucose concentrations, but not yet high enough to diagnose diabetes, facilitating better diagnosis and treatment of the disease.[18]

More and more studies have been published describing the quantification (mainly by real-time qPCR) of microRNAs in Blood (either in plasma or serum) in diabetic patients mainly to detect circulating micro RNA modulation in the chronic form of diabetic complications.[19] In a study conducted in pregnant patients with gestational diabetes (GD), elevated levels of microRNA-330 were observed compared to non-diabetic pregnant women.

Several other studies on microRNA-330-3p expression highlighted that E2F1 (E2F transcription factor 1) is one of the most favorable target transcripts.[20,21] An animal model study highlighted that E2F1/E2F2 compound mutant mice have excessive polyuria, hyperglycemia and decreased blood insulin levels.[22]

E2F1 over expression can stimulate beta cell proliferation. Reduced pancreatic size and increased glucose intolerance due to β-cell dysfunction have been reported in E2F1 – mice.[23] The study evaluated the role of microRNA-330 and E2F1 mRNA regulation in T2DM cases as a suitable traditional biomarker for the diagnosis of various metabolic disorders, including its expression related to T2DM. Indian population primarily to understand the pathogenesis of newly diagnosed type 2 diabetes patients.[15]

Several miRNAs are known to be involved in the pathogenesis of T2DM and their role in diabetic complications has been demonstrated.[24] Kamalden et al. reported that miR-15 plays an important role in insulin secretion in pancreatic cells. In addition, miR-15 has been shown to be produced in pancreatic _cells and enters the circulation, thus contributing to retinal damage during the development of T2DM.[25] The expression of miR-124a is increased in human pancreatic islets with type 2 diabetes, suggesting that miR-124a negatively regulates glucose-induced insulin secretion[26] Literature data indicate that miR-375 regulates glucose homeostasis, insulin secretion, and pancreatic cell development, maintenance, and survival.[27] Most miRNAs are found intracellularly; miRNAs are actively and selectively released by cells in response to stress or injury, so they can act as signalling molecules in health and disease.[28]

The fact that tissue-specific miRNAs can enter the circulatory system, including blood and other body fluids, has opened the possibility of using circulating miRNAs as non-invasive predictors of disease progression.[28] Jimenez-Lucena et al. found that circulating miRNA levels combined with glycated hemoglobin A1c (HbA1c) could be used to predict the development of T2DM.[29]

In addition, miRNAs have also been reported to be associated with prediabetes. For example, the levels of miR-126 and miR-15a were shown to be significantly lower in prediabetes and T2DM.[30,31] However, Kong et al. reported that seven serum microRNAs associated with diabetes (miR-9, miR-29a, miR-30d, miR-34a, miR-124a, miR-146a, and miR-375), which play a key role in differentiating prediabetic patients T2DM-le of susceptible subjects with normal glucose tolerance.[32] Interestingly, many of these miRNAs have also been identified as biomarkers for other metabolic diseases.[33,34] For example, miR-15a-5p and miR-17-5p were determined as predictive biomarkers for MetS.[35] Lin et al. showed an association between urinary miR-29a-3p levels and MetS.[36] In addition, miR-126 has been shown to be a useful marker of metabolic disorders in children with MetS features.[37] A study by Al-Rawaf highlighted that the expression regulation of various miRNAs is related to adipokines and other related metabolic factors in MetS.[38] Elevated miR-122 expression has also been shown in young adults with obesity and insulin resistance.[39] Further studies have shown the role of several miRNAs as attractive potential biomarkers for the treatment of obesity and related risk factor diseases.[40,41]

Positive overexpression of microRNA-330 was observed in diabetic risk factors such as hypertension, obesity, alcoholism and smoking compared to those who were not hypertensive, non-obese, non-alcoholic and non-smokers, showing. relatively lower expression.

Decreased expression of E2F1 was associated with obesity, alcoholism, and smoking, while non-obese, non-alcoholics and smokers had slightly higher expression in comparison. A negative correlation was observed between microRNA-330 and E2F1 mRNA expression among T2DM patients with a p-value of 0.16. Elevated levels of micro-RNA-330-3p may be associated with decreased E2F1 expression and may inhibit beta-cell proliferation and insulin production. Involvement of cell cycle-related genes has previously been shown to be required for beta cell compensation. A 2017 study by Sebastiani G found increased microRNA-330-3p in the plasma of patients with gestational diabetes and suggested that microRNA-330-3p may be an important indicator of GDM outcomes in therapeutic therapy goal.[42]

E2F1 is involved in cell proliferation and metabolism, coordinating the cellular response by acting as a regulatory switch. Studies on the retinoblastoma protein RB1 continue to show an important role in E2F1 metabolism. E2F1 has been shown to be critical for regulating liver metabolism, regulating cholesterol, and promoting lipid biosynthesis through transcriptional regulation of lipogenic enzymes. Increased expression of E2F1 mRNA was detected in liver biopsy samples from diabetic patients, which correlates with PCK1 levels, which is of concern in hyperglycemia in mice and possibly in coding gene regulation. pyruvate dehydrogenase kinase. (PDK4), an important regulator of glucose oxidation and a nutrient sensor that limits mitochondrial glucose oxidation. E2F1 helps regulate metabolic homeostasis through multiple roles in multiple metabolic tissues.

Studies on the retinoblastoma protein RB1 further support the important role of E2F1 in cellular metabolism in humans. However, the function of E2F transcription factors in beta cell pocket proteins is not clearly known. E2F1 plays an important role in hepatic steatosis. E2F1 has been shown to be fundamentally involved in the development of hyperlipidemia and hyperglycemia during insulin resistance, and was abnormally increased in type 2 diabetes Iglesias et al. In 2004, a mouse model showed that E2F1 transcription factors also play an important role in regulating both apoptosis and beta-cell proliferation.[43]

One study showed differential expression of three miRNAs in prediabetic patients who developed T2DM after 5 years compared with prediabetic patients who did not develop T2DM. miR-491-5p, miR-1307-3p and miR-298 are good predictors of T2DM onset years before its onset. ROC analysis was performed to evaluate the diagnostic value of selected miRNAs as predictive biomarkers for T2DM. The highest AUC was observed for miR-491-5p. This increases the potential clinical utility of serum miRNA profiling and highlights the role of miRNAs as potential biomarkers to predict individuals at risk for T2DM. A pooled analysis showed that a logistic regression model consisting of miR-298, miR-1307-3p, and miR-491-5p could show a higher diagnostic accuracy than miR-491-5p alone, suggesting the need to implement such a panel to predict the development of T2DM in prediabetic patients, as the miRNA combination has the highest diagnostic power. In addition, functional analysis of miRNA target genes revealed several biological pathways involved in the development of T2DM. GO analysis revealed that cellular connectivity, transcriptional activation activity and stress fiber assembly play important roles in the mechanism of T2DM development, consistent with hub gene analysis.

The five most common diseases identified by IPA confirmed the effect of the above pathways not only on T2DM but also on muscle autophagy. Currently, muscle autophagy is considered a complication of T2DM rather than a symptom of prediabetes.[44] The ubiquitin-proteasome, autophagy, and proteolytic pathways are involved in protein degradation in muscle, contributing to muscle atrophy.[45] A recent study by Sambashivaiah et al. showed a similarity in skeletal muscle mass, strength and contraction quality between prediabetic and T2DM patients.[46] These results, consistent with the analysis, suggest that muscle atrophy develops years before the development of T2DM. However, the dysregulation of the muscle atrophy pathway in prediabetic patients needs a detailed molecular study to fill the data gaps.[47]

Association of Type I Diabetes with Blood Co-Circulating Extracellular microRNAs and Immune Cell Subsets

One of the most prevalent chronic illnesses is type 1 diabetes. Children are the cause of this and the incidence has increased in children under the age of 15[48]. through the interaction of immunological environmental and genetic elements [49]. The remaining cell and a significant portion of the cell mass have already been destroyed at the time of T1D diagnosis. Over time mass gradually decreases.[50] especially in the absence of appropriate clinical management for each. Patient the advancement of T1D causes major problems that lower life expectancy and quality of life. Instead, knowledge of would significantly enhance the treatment and results for these patients. The underlying mechanisms of the consequences of disease. to assess whether there might be a relationship between the dysregulation of plasmatic miRNAs and. We examined the relationship between peripheral blood immune cells in circulation and T1D. in both CTR and T1D children's plasma between immune cells and miRNAs. Under healthy conditions. strong relationships between peripheral blood circulating

immune cells and plasmatic miRNAs. were discovered to be few and primarily made up of a single edge relation. The relationship between peripheral blood and is being tested in this study as far as we know. The relationship between immune cells and co-circulating miRNAs and the severity and onset of T1D disease. The outcome. findings of the research show that peripheral blood and plasmatic miRNA dysregulation are present in T1D children. Given these parameters there may be a relationship between the imbalance in the circulating T and B lymphocyte subsets. compared to healthy conditions was discovered to be more frequently correlated. Additionally. According to the results some plasmatic miRNAs function better than immune cells in peripheral blood. counts to determine the presence of DKA residual pancreatic function and T1D status. We are unable to determine whether the associated variables are because this study is observational in nature. a direct cause of the pathophysiology of T1D.

Still every miRNA discovered in this investigation was dysregulated. possess a history of being connected to T1D as biomarkers or possibly pathogenic factors. Inside. namely of the seven plasmatic miRNAs that we discovered to be strongly linked to the onset of T1D disease. Four of the proteins in Table 2 (let-7c -7d -7f -7i) are members of the let-7 family which is a central regulator of mammalian glucose. digestion. Reduced glucose tolerance is the outcome of let-7 overexpression on a global and pancreatic level. The inhibition of let-7 results in an insulin-sensitive state that can fend off diabetes caused by a high-fat diet. and treating glucose tolerance impairment is sufficient in this instance.[51,52]

Also let the 7 family. Members control crucial facets of immune cell biology including the determination of lymphocyte cell number. the suppressive effects of extracellular let-7 miRNAs activation function and energy. $CD4^+$ T regulatory cells capacity for action and differentiation[48,53–58]

Circulating miRNA in diabetic retinopathy

One of the most common and devastating microvascular complications that can lead to diabetes is diabetic retinopathy (DR)[59], which affects over 30% of patients. of vision loss in the working-age population.[60,61] It is often the first microvascular complication to appear, and the risk of developing DR is directly related to the duration of diabetes and level of metabolic control.[62]

In recent years, great efforts have been made to identify new biomarkers, and to predict diabetes (especially type 2 diabetes) in individuals at risk of the disease, and to prevent the development of diabetes. long-term complications of diabetes. Another study, investigated the differential expression of circulating miRNAs in a group of patients with type 2 diabetes.

Non proliferative DR. after extensive serum miRNA profiling and validation of individual miRNAs, circulating miRNAs appeared to be upregulated in DR patients compared to non-diabetic diabetics. In particular, one of them, miR-1281, was the most highly regulated and seemed to be most closely related to DR, showing the strongest sensitivity and specificity to detect this microvascular complication of diabetes.

Furthermore, the study results in this work are consistent with other studies showing that miR-1281 is elevated in the central membrane of the cornea of diabetic subjects[63], both in plasma and urine samples from patients with chronic kidney disease, often secondary to poorly controlled diabetes.[64]

High levels of microRNA-15a are associated with an increased risk of amputation after revascularization in T2DM patients with severe peripheral vascular complications.[65] In addition, a recent study showed high levels of extracellular vesicle (EV)-associated microRNA-15a in the plasma of patients with diabetic retinopathy.[66]

Here a pilot observational study provided preliminary evidence for the utility of total and EV-microRNA-15a as an early biomarker of complications in patients with preclinical T2DM.

Circulating microparticle concentrations are reported to be increased in diabetic patients with a specific signature of microRNAs.[67] We have shown here that diabetic EVs are enriched in larger particles, which may reflect increased cell damage in diabetes.[68] Interestingly, we observed a peak of small exosome-like vesicles only in IGT donors, which may be related to inflammation.[69,70,59]

One study provided preliminary evidence that patients with T2DM and DR present distinct changes in exosome-mediated circulating miRNAs. In particular, it was observed higher expression of miR-25-3p and miR-320b and lower levels of miR-495-3p compared to non-diabetic subjects and patients with T2DM without DR. Interestingly, the expression of these miRNAs correlates with disease severity. These associations were independent of age, gender and glycemic control (reflected in circulating HbA1c), and simultaneous evaluation of the three miRNAs provided good accuracy in classifying patients with DR in this study.

Few studies have previously investigated the expression of miRNAs in diabetic patients with DR. A large, nested case-control study investigated the expression of 29 circulating miRNAs in two prospective cohorts of patients with T1DM (PROTECT-1 and PREVENT-1). The associations of miR-27b-3p and miR-320a-3p with the prevalence of DR14 were used in this study. In this study, another member of the miR-320 family (namely miR-320b) was significantly increased, while changes in miR-320a-3p did not reach statistical significance. In particular, although miR-27b-3p and miR-320a-3p were highly expressed in endothelial cells and may affect

angiogenesis14, the cellular ontology of the FANTOM5 database suggests that the miRNAs identified in this study are enriched in other cells types (mainly fibroblasts, stem cells and leukocytes).

In another study, investigation of circulating miRNAs that are transported by vesicles was done, including exosomes. Exosomes are of particular interest in biology because their biogenesis involves helical intracellular protein complexes that selectively produce miRNAs and other cargoes. Previous studies show that exosomes can effectively deliver miRNAs to recipient cells to influence the biological response of the paracrine/endocrine communication system. Previously, only Mazzeo et al.23 investigated miRNAs carried by exosomes in DR patients who showed dysregulation of miR -150-5p, miR-21-3p and miR-30b-5p and clearly demonstrated in vitro functional relevance in important pathophysiological features of DR. Although Mazzeo et al. studied patients, \insulin-treated T1DM, their results support the existence of a regulatory pathway of vesicular miRNAs in DR.

In particular, the validated targets of miRNAs identified in this study are associated with pathways important in the pathogenesis of DR, including regulation of response to growth factors, metabolic processes, cell differentiation, stress response and vascular development. Thus, it is possible that dysregulated miRNAs reflect active intracellular communication that leads to (or compensates for) the development of DR.[71]

Gestational diabetes and its relation with Circulating micro RNA

Gestational diabetes (GDM) is one of the most common diseases during pregnancy. According to the latest edition of the Diabetes Atlas of the International Diabetes Federation (IDF), GDM affected almost 17 million live births last year. Vast hormonal changes during pregnancy are one of the causes of increased insulin resistance. Immediately the hyperestrogenic state observed during pregnancy contributes to changes in insulin sensitivity. Estrogen can bind directly to insulin or its receptors, making them inaccessible to insulin. In addition, human placental lactogen (hPL) reduces maternal insulin sensitivity to ensure adequate nutrition for the fetus. If insulin release is insufficient and a glucose-lowering response is not achieved, the risk of developing GDM is high. A meta-analysis showed that high BMI and thyroid disease are the main risk factors for GDM. Other risk factors include elevated fasting blood glucose during the first trimester of pregnancy, abdominal obesity, family history of diabetes, genetic factors, environmental factors including lifestyle and diet, and comorbidities such as polycystic ovary syndrome (PCOS). Combinations of multiple risk factors are more likely to identify women at high risk for GDM. Considering that the utility of risk factors such as first trimester fasting blood glucose monitoring is limited, the search for an ideal non-invasive biomarker for early detection of GDM or even tendency to develop GDM is necessary. It is of interest to have a GDM biomarker that shows up before any glycaemic changes take place, taking into account the high risk of complications during pregnancy and delivery and the potential for long-term complications for both the mother and baby. Yoffe et al. studied women at 9 and 11 weeks of gestation and showed upregulation of miR-223 and miR-23a in the plasma of GDM women. An interesting perspective was presented by Wander et al. Association of miR-21-3p and miR-210-3p with GDM diagnosed in overweight and obese women. Lamadrid-Romero et al. showed that miR-183-5p increased every trimester in serum collected from women diagnosed with GDM. Meanwhile, higher expression of miR-125b-3p, miR-200b-3p and miR-1290 was observed in the first trimester of pregnancy. Another study shows that circulating miR-16-5p is upregulated in women before the onset of GDM, which is consistent with the results of other studies. Zhu et al.conducted studies in women between 16 and 19 weeks of gestation and described five molecules that were upregulated in the GDM group (eg, miR-16-5p). Other studies reported increased expression of miR-16-5p in serum at 24-28 weeks of gestation. The results show this difference earlier between 9 and 12 weeks of pregnancy. In addition, we observed a positive correlation with HOMA-IR, also reported by Cao et al. The plasma of healthy pregnant women contained high levels of MiR-574-5p and miR-315b, whereas their expression was dysregulated in those with GDM. The GO and KEGG pathway enrichment of these two miRNA target genes were further analyzed.[72]

Circulating miRNA in patients undergoing treatment of diabetes

This study investigated the expression of circulating miRNAs (miR-30a-5p, miR-1299, miR-182-5p, miR-30e-3p and miR-126-3p) in newly diagnosed and known diabetic patients. Treatment this study data show that these miRNAs are differentially expressed in individuals with diabetes and in newly diagnosed and treated individuals. All target miRNAs were significantly correlated with each other except miR-1299 and miR-126-3p. Correlations were illustrated between miR-30a-5p and miR-30e-3p with fasting blood glucose and HbA1c and triglyceride-S. In addition, all miRNAs except miR-1299 were significantly correlated with HDL cholesterol and total serum cholesterol, as well as inflammatory markers (usCRP and γ-glutamyl transferase), and similar associations were observed with measures of renal function, MDRD eGFR and CKD-EPI. We found that disease duration is the most determining factor in the expression of these miRNAs. For example, miR-182-5p was significantly decreased over 8 years in subjects with diabetes, but no such differences were observed when antidiabetic treatment was taken into account. In addition, miR-1299, miR-182-5p, and miR-126-3p were significantly associated with T2DM in age- and sex-adjusted regression analysis, but this association disappeared for miR-1299 and miR-1299 when drugs

were included. in the model. miR. -126-3p and only miR-182-5p are conserved. This study showed significant associations and altered expression patterns of miR-30a-5p, miR-1299, miR-182-5p, miR-30e-3p and miR-126-3p in diabetic patients with antidiabetic treatment versus recent treatment diagnosed cases. In addition, we show that miR-182-5p particularly decreases as the duration of T2DM increases. Longitudinal and functional studies are recommended to elucidate miRNA involvement in insulin signalling and glucose homeostasis to support its use as a therapeutic target in DM and related complications.[73]

CONCLUSION

The results indicate that microRNAs can be introduced as a diagnostic tool for the prediction of T2DM. These data's show that candidate miRNAs deregulated years before T2DM development. More research are needed for understanding the role of circulating miRNAs in the molecular mechanisms underlying T2DM and T1DM and for identifying at-risk individuals.

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